

The Claims Defining the Invention are as Follows

1. A host-cell free method for culturing *Cryptosporidium* comprising the step of introducing *Cryptosporidium*, at a first lifecycle stage, into a host-cell free medium under conditions which enable the *Cryptosporidium* to progress to a second lifecycle stage.
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2. A method according to claim 1 wherein the first and second lifecycle stages are selected from the group consisting of: oocyst including excysted oocysts, sporozoite, trophozoite, meront I, merozoites (Type 1), meront II (early), meront II (late), merozoites (type II), macrogamont, microgamete and zygote.
- 10 3. A method according to claim 1 wherein the first lifecycle stage is an oocyst or a sporozoite and the second lifecycle stage is an oocyst, sporozoite or a trophozoite.
4. A method according to claim 1 wherein the second lifecycle stage is an oocyst.
5. A host-cell free method for culturing *Cryptosporidium* comprising the step of
15 introducing *Cryptosporidium*, at a first lifecycle stage, into a host-cell free medium under conditions which enable the *Cryptosporidium* to complete its lifecycle.
6. A host-cell free method for producing *Cryptosporidium* biomass from an initial inoculum of *Cryptosporidium* comprising the steps of: (i) putting the inoculum
20 into a host cell free medium; and (ii) culturing the *Cryptosporidium* to increase the *Cryptosporidium* biomass.
7. A method according to any one of the preceding claims wherein the host cell free medium is a buffered and balanced combination of inorganic salts, amino acids and vitamins.
- 25 8. A method according to claim 7 wherein the medium further comprises an additional constituent selected from the group consisting of: a carbohydrate source, antibiotics, bile and serum.

- 26 -

9. A method according to any one of the preceding claims wherein the medium has a pH at or about neutral pH.
10. A method according to any one of the preceding claims wherein the host cell free medium further comprises a second phase in the form of serum that has
5 been treated to render it viscous or semi-solid.
11. A method according to claim 10 wherein the serum is coagulated.
12. A method according to claim 10 or 11 wherein the serum used to form the second phase is foetal calf serum.
13. A host-cell free method for culturing *Cryptosporidium* comprising the steps of:
- 10 a. isolating *Cryptosporidium* oocysts;
b. excysting the isolated oocysts;
c. resuspending the excysted oocysts in a host-cell free culture medium;
d. incubating the culture prepared in step (c) under suitable conditions;
and
15 e. harvesting oocysts from the medium.
14. A method according to any one of the preceding claims wherein the *Cryptosporidium* belongs to the species selected from the group consisting of:
Cryptosporidium andersoni, *Cryptosporidium parvum*, *Cryptosporidium muris*,
Cryptosporidium hominis, *Cryptosporidium wrairi*, *Cryptosporidium felis*,
20 *Cryptosporidium canis*, *Cryptosporidium baileyi*, *Cryptosporidium meleagridis*,
Cryptosporidium galli, *Cryptosporidium serpentis*, *Cryptosporidium saurophilum* and *Cryptosporidium molnari*.
15. A host cell free medium capable of maintaining *Cryptosporidium* or enabling the progress of *Cryptosporidium* through its lifecycle, the medium comprising a
25 buffered and balanced combination of inorganic salts, amino acids, vitamins and additional constituents.
16. A biphasic host cell free medium capable of maintaining *Cryptosporidium* or enabling the progress of *Cryptosporidium* through its lifecycle the medium

- 27 -

comprising a buffered and balanced combination of inorganic salts, amino acids, vitamins and additional constituents.

17.A medium according to claim 15 or 16 wherein the additional constituents are selected from the group consisting of: amino acid supplements, carbohydrate
5 source, antibiotics, bile and serum.

18.A medium according to any of claims 15 to 17 with a pH about neutral.

19.A medium according to claim 16 wherein the second phase comprises serum that has been treated to render it viscous or semi-solid.

20.A medium according to claim 19 wherein the serum is foetal calf serum.

10 21.A method for preparing an immunogenic preparation comprising at least one *Cryptosporidium* antigen, the method comprising the steps of: (i) introducing *Cryptosporidium*, at a first lifecycle stage, into a host-cell free medium under conditions which enable the *Cryptosporidium* to progress to a second lifecycle stage; (ii) isolating the *Cryptosporidium* at the second lifecycle stage; and (iii)
15 preparing a therapeutic preparation using the *Cryptosporidium* isolated from step (ii).

22.A method according to claim 21 wherein the second lifecycle stage is an extracellular lifecycle stage.

23.A method according to claim 21 wherein the second lifecycle sage is a
20 trophozoite, merozoite or other extracellular gamont-like stage.

24.A therapeutic composition comprising a therapeutically effective amount of *Cryptosporidium* cultured according to any one of claims 1 to 14 and a physiologically acceptable carrier.

25.A composition according to claim 24 comprising a whole cell extract of one or
25 more *Cryptosporidium* lifecycle stages.

26.A composition according to claim 25 comprising one or more *Cryptosporidium* lifecycle stages that have been treated to disrupt their cellular structure.

- 28 -

- 27.A composition according to claim 24 comprising at least one isolated and purified *Cryptosporidium* antigen.
- 28.A composition according to claim 26 wherein the cellular disruption has been achieved by a technique selected from the group consisting of: sonication,
5 osmotic pressure, freezing, exposure to detergents such as sodium dodecyl sulfate (SDS), and heating.
- 29.A composition according to any one of claims 24 to 28 wherein the *Cryptosporidium* cells have been inactivated.
- 30.A method of preventing or treating a disease associated with *Cryptosporidium*
10 infection in a subject comprising administering to the subject a therapeutically effective amount of a composition according to any one of claims 24 to 29.
- 31.A method for detecting *Cryptosporidium* in a sample comprising the steps of:
(i) subjecting the sample to the culture method described herein; and (ii) detecting the *Cryptosporidium*.
- 15 32.A method for detecting *Cryptosporidium* in a sample comprising the steps of:
(i) introducing the sample into a host-cell free medium under conditions which enable *Cryptosporidium* to progress to a further lifecycle stage; and (ii) detecting the *Cryptosporidium*.
- 20 33.A method for detecting *Cryptosporidium* in a sample comprising the steps of (i) introducing the sample into a host-cell free medium under conditions which enable the *Cryptosporidium* to complete its lifecycle; and (ii) detecting the *Cryptosporidium*.
- 34.A method according to any one of claims 31 to 33 wherein the sample is from a water source that is to be used by humans or animals.
- 25 35.A method according to claim 34 wherein the water source is a source of drinking water such as a dam, lake, river or rain catchment area.
- 36.A method according to any one of claims 31 to 35 wherein the *Cryptosporidium* is detected via visual examination.

- 29 -

37.A method according to claim 36 wherein the visual examination is via a microscope or some other means that enables any *Cryptosporidium* in the sample to be viewed.

38.A method according to any one of claims 31 to 35 wherein the
5 *Cryptosporidium* is detected using PCR.

39.A method according to any one of claims 31 to 38 further comprising the step of pretreating the sample to concentrate any *Cryptosporidium* therein.

40.A method according to claim 39 wherein the pre-treatment comprises centrifugation of the sample.

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